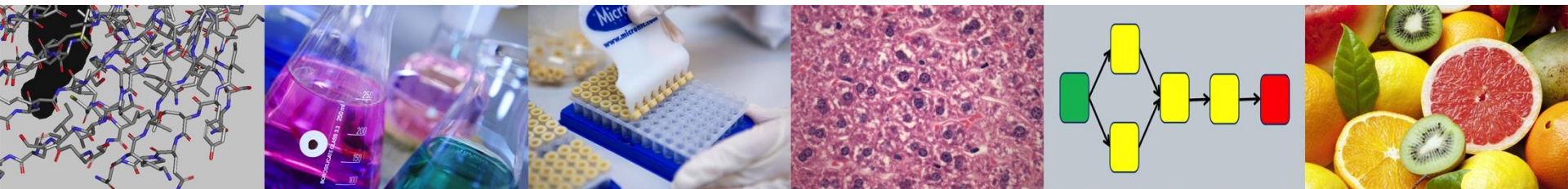




AOP based approach for mixture testing and risk assessment by the EuroMix project

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EuroMix – European Horizon 2020 project

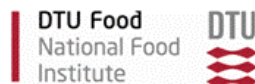
22 partners from 16 countries, linked to international organisations including WHO, FAO and EFSA.
EuroMix is coordinated by Jacob van Klaveren, RIVM, Netherlands.



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EuroMix handbook

- Methodology and tools for mixture risk assessment
- Examples

EuroMix toolbox

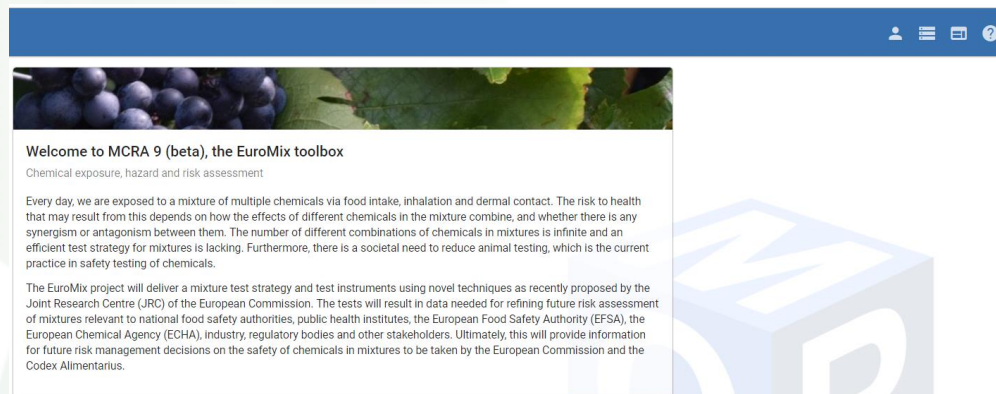
- Web based toolbox for mixture risk assessment
- Data repository



EuroMix handbook for mixture risk assessment

Draft April 2, 2019

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Mirjam Luijten and Jacob van Klaveren, RIVM, The Netherlands
Hilko van der Voet, Wageningen University & Research, Biometris, The Netherlands
and additional authors to be included



EuroMix handbook and the EuroMix toolbox provide practical support to apply the recent OECD and EFSA guidance documents in mixture risk assessment



Component-based approach

- Toxicity and exposure data on individual substances in the mixture
- Predict toxicity of mixture

Dose addition

- Substances with similar toxicity
- Substances in mixtures treated as dilutions of each other scaled for the potencies
- Default, conservative model

Assessment groups

- Grouping based on toxicological considerations

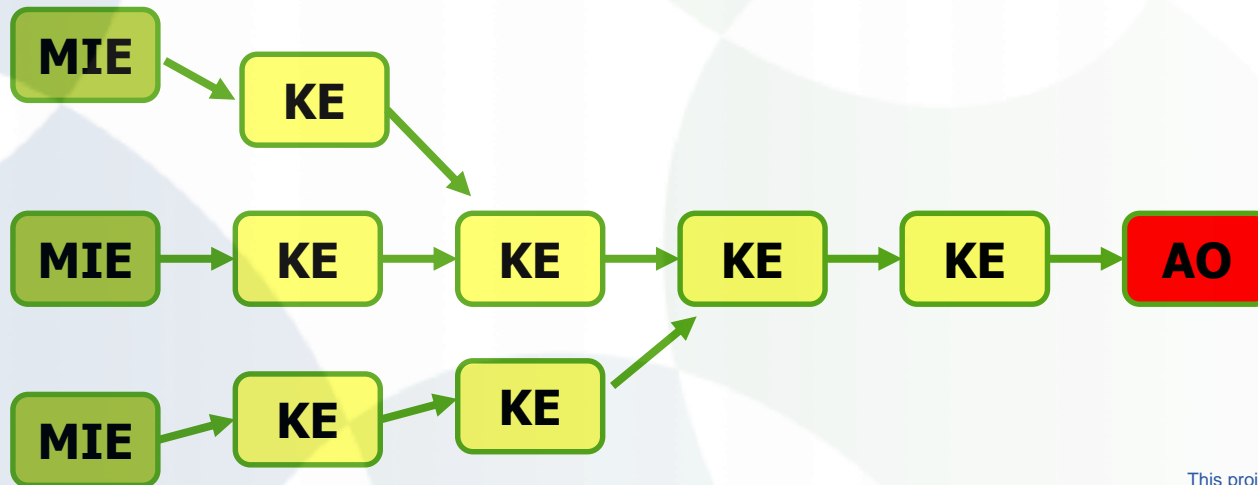
Toxicity data for mixture risk assessment

Toxicity data needed for

- Grouping into assessment groups
- Potency information (relative potency factors)
- **In vivo data**
 - Not always available or feasible to produce for all substances
- **In vitro data**
 - Inform grouping
 - Relative potency factors using in vitro to in vivo extrapolation (IVIVE)
 - Tiered testing strategies and set priorities for in vivo testing
- **In silico data**
 - Inform grouping
 - Tiered testing strategies and set priorities for in vitro testing

AOP networks for mixture risk assessment

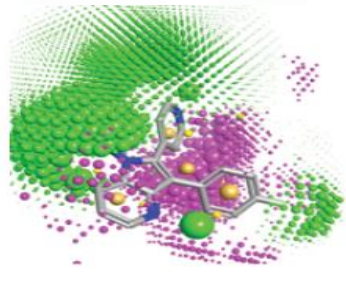
- Identify any existing AOPs
- Develop new AOP starting from AO
- Identify KEs and KE relationships
- Focus on easily measured KEs
- Complete AOP not necessary
- Assess the postulated AOP



Tiered testing strategy based on AOP networks

- Identify KEs that can provide info for grouping or RPFs in the AOP network
- Identify in silico, in vitro and in vivo assays for the KEs
- Assess the
 - relevance of the assays
 - reliability of the assays
 - availability and feasibility in terms of costs and resources
 - information provided for grouping, RPFs, prioritisation for further testing
- Select assays to be included

In silico



In vitro



In vivo



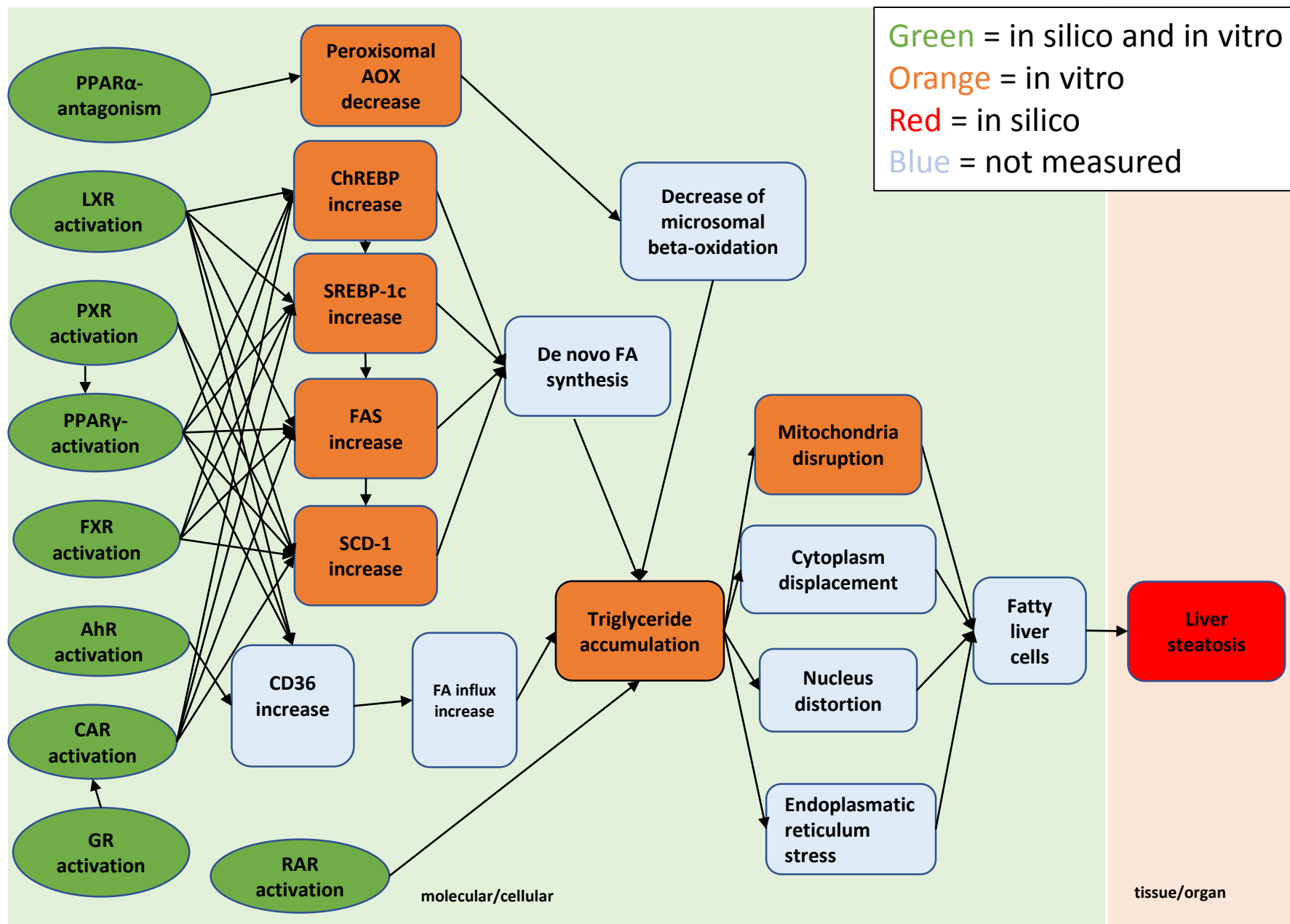
This project is funded by the Horizon
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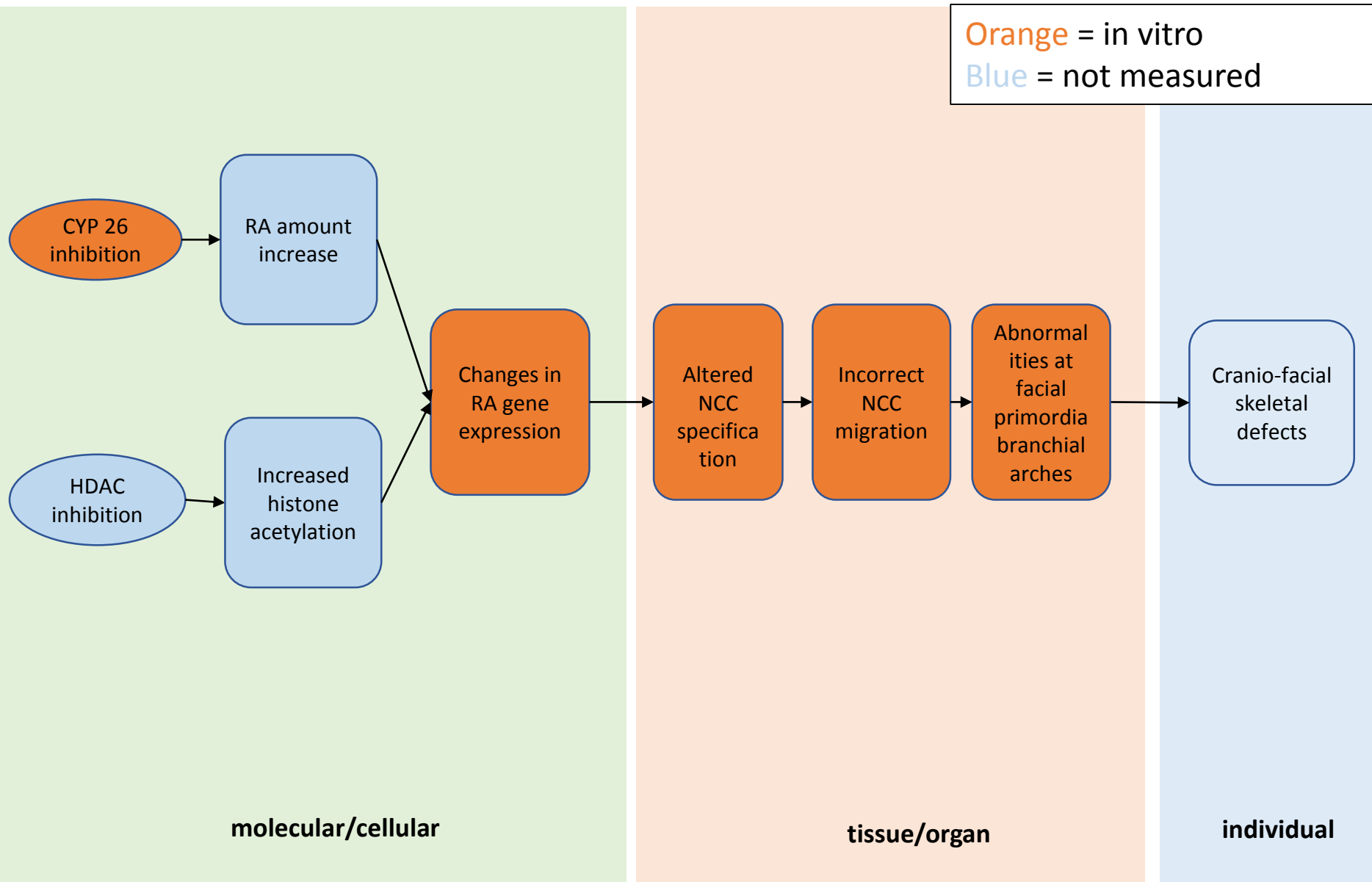
Template for tiered testing strategy

KE number in AOP network	KE name	<i>In silico</i> model/ <i>in vitro</i> assay for measuring the KE	Relevance of the <i>in silico</i> model/ <i>in vitro</i> assay	Reliability of the <i>in silico</i> model/ <i>in vitro</i> assay	Availability and feasibility of <i>in silico</i> model/ <i>in vitro</i> assay	Information provided by the <i>in silico</i> model/ <i>in vitro</i> assay for the mixture risk assessment (e.g. for grouping, RPFs and/or prioritisation for further testing)
MIE1						
MIE2						
KE1						
KE2						
KE3						
KE4						
KE5						
KE6						
KE7						
AO						

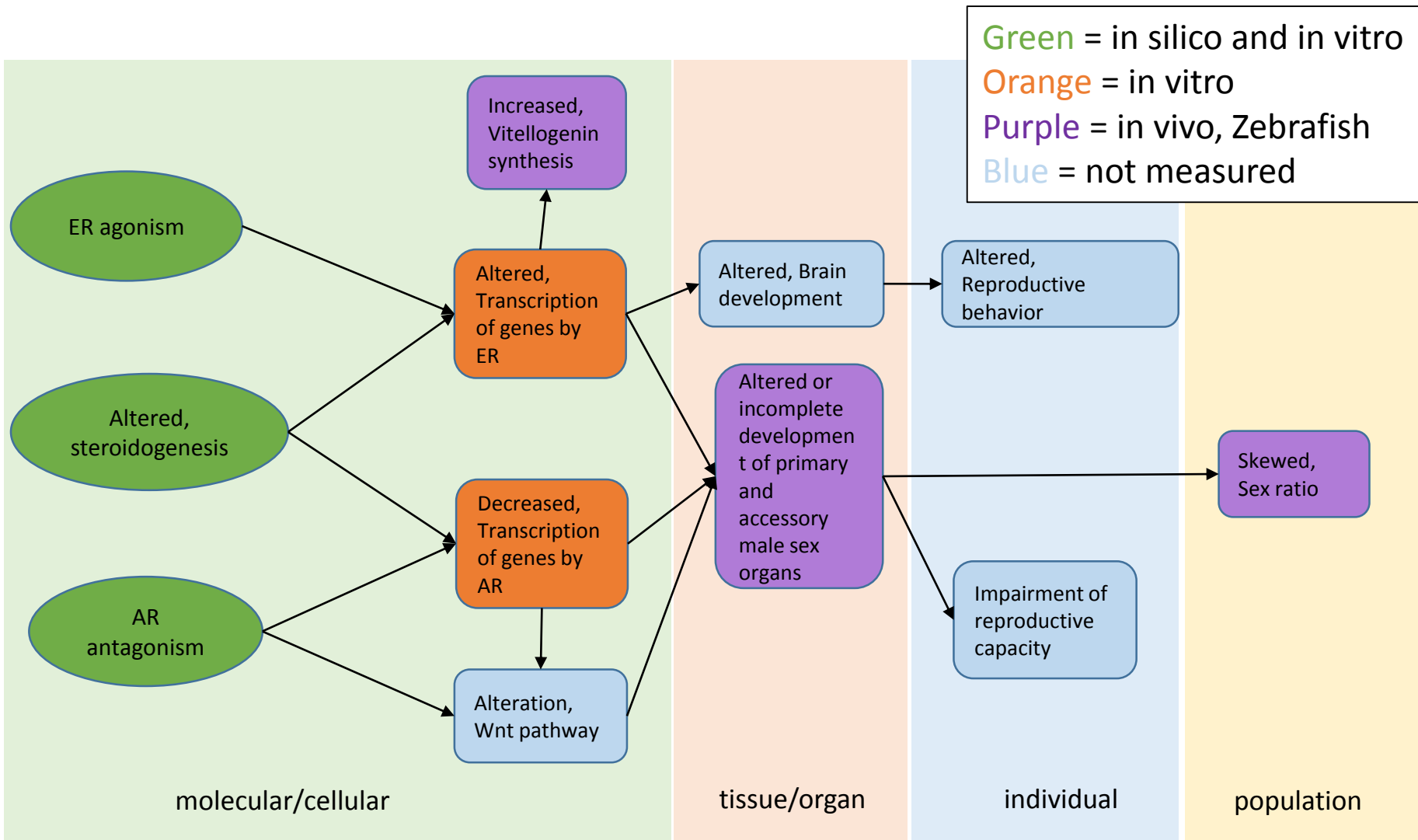
AOP based testing strategy for liver steatosis



AOP based testing strategy for craniofacial malformations



AOP based testing strategy for estrogen/antiandrogen balance and reproductive toxicity



Grouping of substances based on toxicological considerations

Methodology

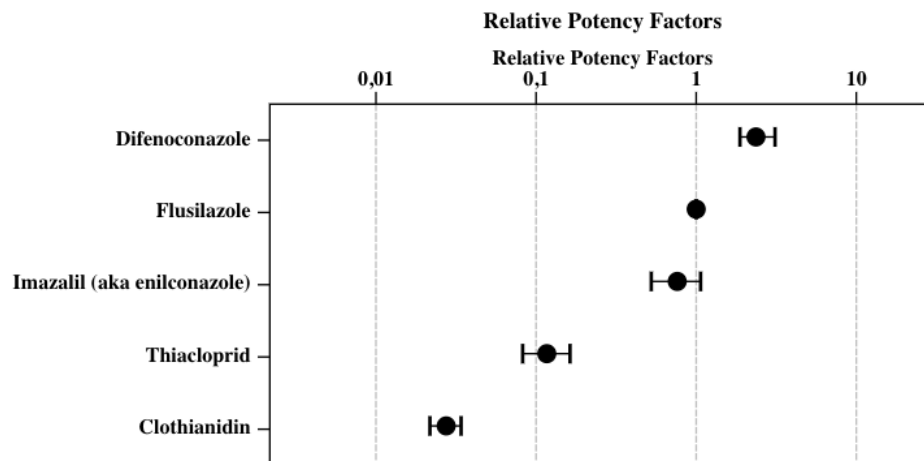
- Level of grouping (target organ, common effect/AO, common specific mode of action/AOP)
- AOP network
- Substance category
- Collect toxicity data (in silico, in vitro, in vivo, human)
- Organise data in lines of evidence
- Assess data for relevance and reliability
- Decide on group membership using weight of evidence approach

Template for organising data for grouping

Substance	Key event in the AOP network (organised according to MIE, intermediate KEs, AO)	Study type (organised according to <i>in silico</i> , <i>in vitro</i> , <i>in vivo</i> data, human study)	Assay (specific assay used)	Main study result (e.g. positive, negative, BMDL, NOAEL)	Reliability (low, medium, high)	Relevance (low, medium, high)
	MIE	<i>In silico</i>				
		<i>In vitro</i>				
		<i>In vivo</i>				
		Human				
	Each intermediate KE	<i>In silico</i>				
		<i>In vitro</i>				
		<i>In vivo</i>				
		Human				
	AO	<i>In silico</i>				
		<i>In vitro</i>				
		<i>In vivo</i>				
		Human				

Potency information (relative potency factors)

- Data sources for RPFs
 - NOAELs/BMDs from in vivo studies in literature
 - Experimental in vitro and in vivo dose response data from the AOP based testing strategy



In vitro to in vivo extrapolation (IVIVE) is needed to use the in vitro RPFs in the dietary exposure assessment

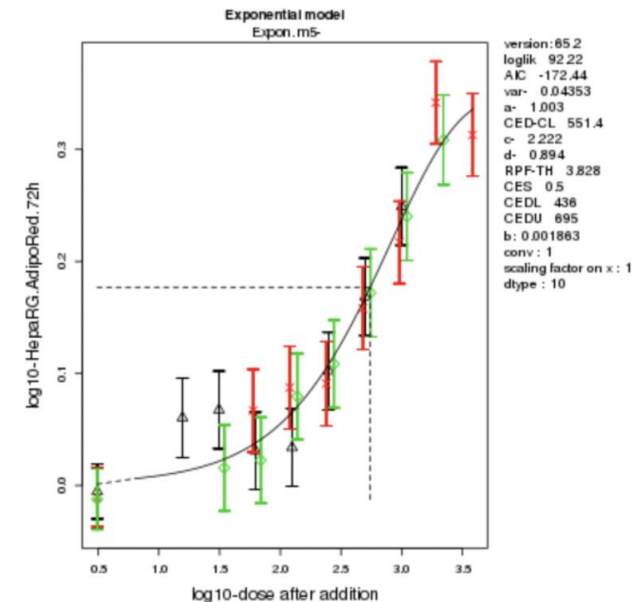
Mixture testing

Is the binary mixture dose additive?

Are there interactions: synergism or antagonism?

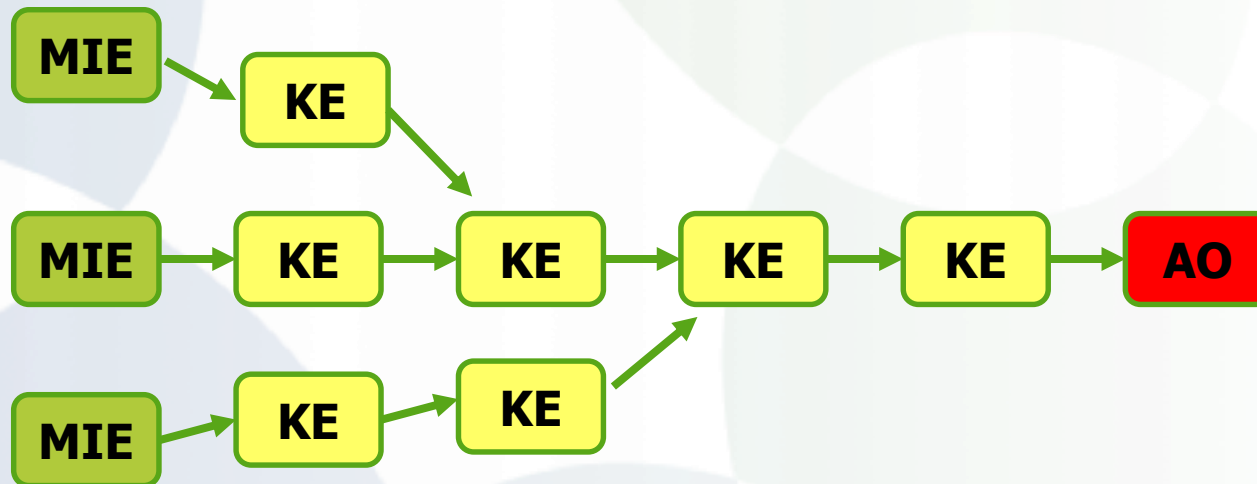
- Equal potency of substances
- RPFs of individual substances needed
- Several doses of individual substances and binary mixture
- Results analysed using benchmark dose method

Black triangles and red crosses: single substances
Green diamonds: mixture



Conclusions

- AOP based approach for mixture testing and risk assessment provide support to generate and identify toxicity data for
 - grouping of substances
 - potency information
 - mixture testing



Acknowledgements



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- Alfonso Lampen, Leo van der Wen, Toine Bovee, Angelo Moretto, Elena Menegola, AOP networks and in vitro testing strategies



<https://www.euromixproject.eu>

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